

ACTIVATION OF ADENOSINE 5' TRIPHOSPHATE-INDUCED QUENCHING OF CHLOROPHYLL FLUORESCENCE BY REDUCED PLASTOQUINONE

The basis of state I—state II transitions in chloroplasts

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Received 22 July 1980

1. Introduction

The distribution of absorbed radiation arriving at the reaction centres of PSII and PSI in chloroplasts is subject to regulation [1,2]; this phenomenon was first defined in terms of the state I—state II transitions, whereby light absorbed by PSII causes a change that enhances the efficiency of PSI and light absorbed by PSI induces a reversal of this change [3]. Experimentally, this process has been studied by observing the slow quenching of chlorophyll fluorescence that occurs during a period of several minutes following illumination [4–6]. At least part of this quenching is due to the establishment of a transmembrane proton gradient [4–8] but it has been shown that such quenching does not elicit a change in exciton distribution between PSII and PSI [8,9]. In isolated chloroplast membranes addition of ATP causes a 25–30% quenching of chlorophyll fluorescence [9,10] after 10 min incubation. This quenching is light-dependent, uncoupler insensitive but inhibited by DCMU. Fluorescence emission spectra at -196°C indicate an increase in the fraction of energy transferred to PSI after ATP treatment. It was suggested therefore that this effect could be the basis for physiological regulation of exciton distribution. The molecular mechanism for the ATP-induced quenching is the presence in

chloroplast membranes of a light-activated protein kinase which can, when activated, phosphorylate the major LHC [10–12], thus altering its energy-transferring properties. Light activation was assumed to require photosynthetic electron transport since DCMU inhibited activation [9,10]; because electron donors to PSI did not overcome DCMU inhibition, it was suggested that the reduction of plastoquinone is necessary for the activation of the protein kinase [9]. Here we show that:

- (i) Red and far-red light antagonistically affect activation of the ATP-induced fluorescence quenching;
- (ii) Dark activation can be induced by chemical reduction;
- (iii) Reduction of a component with $E_{m7.8} = +55\text{ mV}$ is involved. It is concluded that the redox potential of plastoquinone determines exciton distribution in chloroplasts.

2. Materials and methods

Chloroplasts were isolated from 12–16 day-old peas essentially as in [13] but incorporating the following modifications. The grinding medium contained 0.33 M glucose, 50 mM $\text{Na}_2\text{-HPO}_4$, 50 mM KH_2PO_4 , 5 mM MgCl_2 , 25 mM NaCl, 0.2% sodium iso-ascorbate and 0.1% bovine serum albumin, adjusted to pH 6.5. Initial purification was performed by squeezing the leaf homogenate through 2 layers of muslin followed by filtration through 8 layers of cotton wool. After centrifugation, pellets were rinsed with 0.33 M glucose, 5 mM MgCl_2 , 26 mM sorbitol,

Abbreviations: PSI, photosystem I; PSII, photosystem II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid; Tricine, *N*-tris-(hydroxymethyl)methylglycine; LHC, light-harvesting complex; Q, primary electron acceptor of PSII; E_m , midpoint oxidation reduction potential; E_h , oxidation reduction potential, $E_h = E_m + \frac{RT}{F} \ln \frac{(\text{ox})}{(\text{red})}$

4 mM Hepes (pH 7.6) and gently resuspended in 0.33 M sorbitol, 4 mM EDTA, 5 mM $MgCl_2$, 2 mM $MnCl_2$ and 100 mM Hepes (pH 7.6). The reaction medium for all experiments contained 0.1 M sucrose, 10 mM NaCl, 3 mM $MgCl_2$ and 10 mM Tricine, adjusted to pH 7.8, with chloroplasts at 10 or 15 μg chl./ml. Measurement of chlorophyll fluorescence was made at 90°C to the excitation beam in a 1 × 1 cm thermostatted and stirred cuvette, emission being defined by a Balzers 694 nm interference filter and measured by an EMI 9558B photomultiplier tube. Excitation was by means of a tungsten-halogen lamp powered at 100 W and filtered as in table 1. Fluorescence emission spectra at -196°C were recorded front face on samples of chloroplasts 1 mm thick. Excitation was defined by a 4-96 Corning filter and was chopped at a frequency of 720 Hz using a Rofin microchopper. Emission was detected through an Oriel monochromator with a resolution half-bandwidth of 2 nm and the photomultiplier (EMI 9558 QB) output was measured using an EG and G Brookdeal 9503 Lockin amplifier.

Redox titration was performed essentially as in [14] except that an EIL miniature combination platinum electrode was used in a redox cell that required only a 3 ml sample.

3. Results and discussion

Table 1 shows the extent of ATP-induced quenching of chlorophyll fluorescence induced by different light regimes. Saturating blue light causes 22% quenching, the same value as in [9]. In this experiment, the phosphatase inhibitor NaF [12] was not used and hence this value is less than the maximum 25–30% obtainable. Low intensity red light causes 15% quenching, or ~66% of the saturating light value. In contrast, far red light of the same intensity induces only 2% quenching. Successive treatment with far red followed by red appears to give an additive effect (18%), to be expected under nonsaturating conditions. However, if red light is given first followed by far red only 8% quenching is observed; thus far red illumination (exciting PSI only) can substantially reverse the effect of red light (exciting both systems, but PSII preferentially).

The antagonistic effects of red and far red light upon the activation of the ATP-induced quenching suggests that a component of the intersystem elec-

Table 1
ATP-induced fluorescence quenching in different light regimes

| Light regime | ATP-induced quenching % |
|-------------------|-------------------------|
| (a) Dark | 0 |
| (b) Far red | 2 (0–3) |
| (c) Red | 15 (13–17) |
| (d) Far red + red | 18 (16–20) |
| (e) Red + Far red | 8 (6–10) |
| (f) Blue | 22 (20–24) |

Chloroplasts were incubated in the presence and absence of ATP under the conditions shown. Saturating blue light was defined by a Corning 4-96 filter giving an intensity of 95 W/m^2 . Far red (730 nm Balzers interference filter) and red (650 Balzers interference filter) were each 1.7 W/m^2 . The incubation medium contained ionophore A23187 to inhibit energized quenching, chloroplasts at 10 μg chl./ml and ATP at 0.15 mM. After 10 min of each light treatment (20 min for (d) and (e)) the maximum fluorescence intensity was recorded. Values are means of 4 separate expt. with ranges given in brackets

tron transfer chain is implicated. It seems unlikely that components close to PSI are involved (P700, cytochrome *f* and plastocyanin) since it is PSI light causing oxidation that is the change from the dark-adapted state; red light would only tend to decrease the degree of oxidation. Inhibition of activation by DCMU, when all these carriers would become more oxidized is also inconsistent with their involvement. In addition, the fact that electron acceptors such as methyl viologen prevent activation [9] suggest that it is the reduction of carrier rather than an oxidation that causes activation. Q and plastoquinone are the only components known to be oxidized in darkness but reduced by PSII and re-oxidized by PSI [15]. Again, DCMU inhibition seems to discount Q, leaving plastoquinone as the most likely to be involved.

The involvement of plastoquinone was tested directly by redox titration. It was found that chemical reduction can cause an ATP-induced quenching of ~27% after incubation in complete darkness. Potentiometric titration of this ATP-dependent quenching showed that reduction of a component with $E_{m7.8} = +55$ mV ($\eta = 2$) was responsible for activation of the ATP effect (fig.1). The reported value for the E_{m7} of plastoquinone is +106 mV (also $\eta = 2$) which at pH 7.8 would be +58 mV [17]. Note that ~5% quenching remains at the end-point of the titration. This residual activation can be removed upon further

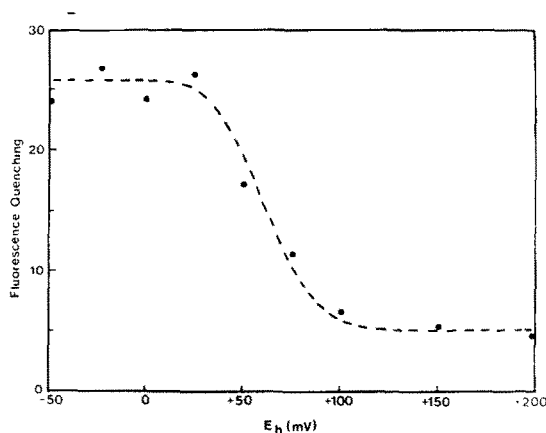


Fig.1. Redox titration of ATP-induced fluorescence quenching. Chloroplasts were incubated at the redox potentials shown for 10 min in darkness and their maximum fluorescence levels recorded. Data is expressed as $[F(-\text{ATP}) - F(+\text{ATP})]/F(-\text{ATP}) \times 100$. The $F(-\text{ATP})$ value decreased by $\sim 5\%$ as the potential was increased from -50 mV to $+200$ mV, presumably due to quenching by oxidized plastoquinone [19]. Redox mediators used were diaminodurene and indigo-tetrasulphonic acid both at $10 \mu\text{M}$. The incubation medium contained 10 mM NaF , $1 \mu\text{g/ml}$ ionophore A23187, and chloroplasts at $10 \mu\text{g chl/ml}$. ATP where added was 0.15 mM . (---) Standard Nernst equation for a 2 equiv. component (i.e. $\eta = 2$) with $E_{m7.8} = +55 \text{ mV}$.

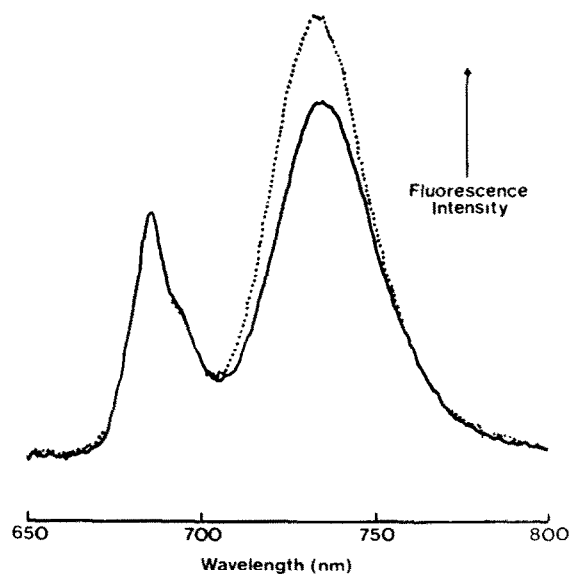


Fig.2. Fluorescence emission spectra at -196°C . Chloroplasts were incubated at -50 mV as in fig.1. After 10 min samples were taken by syringe and rapidly frozen ($\sim 10 \text{ s}$) in a pre-cooled cuvette. Spectra are normalized to the same fluorescence intensity at 685 nm since absolute amplitudes vary because of unavoidable variations in freezing pattern: (—) $-\text{ATP}$; (\cdots) $+\text{ATP}$.

Table 2
ATP-induced change in fluorescence emission at -196°C

| E_h (mV) | F685/F735 | |
|---------------|------------------|------------------|
| | +ATP | -ATP |
| -50 | 0.52 (0.51–0.54) | 0.69 (0.65–0.73) |
| $+200$ | 0.70 (0.65–0.74) | 0.71 (0.71–0.72) |

Chloroplasts were treated as in fig.1 and samples taken for recording of emission spectra at -50 mV and $+200 \text{ mV}$ as in fig.2. Data are the means of 6 (at -50 mV) or 4 (at $+200 \text{ mV}$) expt. with ranges given in brackets

oxidation to $\sim 3\text{--}400 \text{ mV}$. If it is assumed that activation is due to reduction of the protein kinase itself by reduced plastoquinone, then it is possible that this 5% represents a small amount of direct activation by the redox agents in the solution.

Thus, the value of the E_m , the fact that it is a 2 equiv. redox component and the antagonistic effects of PSII/PSI all suggest strongly that it is reduced plastoquinone that activates the ATP effect on room temperature fluorescence. That the ATP-dependent quenching induced by dark reduction of plastoquinone is accompanied by an increase in the fraction of absorbed energy transferred to PSI is shown by examination of the fluorescence emission spectra at -196°C (fig.2, table 2). At -50 mV , but not at $+200 \text{ mV}$, ATP causes a decrease in the F685/F735 ratio, indicative of increased excitation of PSI [16]. Similar changes in this ratio have been reported during light activation of the ATP-induced quenching of room temperature fluorescence [9,10].

The redox state of plastoquinone provides the chloroplast with a sensitive measure of the state of the electron-transfer chain. Thus, any imbalance between the rates of PSII and PSI excitation will be reflected in the redox state of the plastoquinone pool; over excitation of PSII will reduce the pool, activate the protein kinase and, by phosphorylation of LHC, bring about increased excitation of PSI. Hence state I—state II transitions can be easily explained. Furthermore, the redox state of plastoquinone will also respond to other internal as well as external factors. Hence depletion of NADP would trigger, by this process, increased excitation of PSI, possibly thereby enhancing the rate of ATP production by cyclic electron-transfer; this may well also promote dissipation of excess energy by photochemical electron transfer and non-radioactive decay due to the transmembrane

pH gradient [8]. This kind of mechanism could therefore control the ATP/NADPH ratio and may be important in overcoming metabolic imbalances of the kind in [18]. Finally, the redox state of plastoquinone will also respond to the levels of adenylates and to light intensity. A successful control mechanism is made possible by the presence in the chloroplast membrane of a phosphatase that dephosphorylates the LHC [12], so that the system will be able to respond quickly to oxidative changes in the plastoquinone pool.

In conclusion, there is now considerable evidence to support the viewpoint that regulation of photosynthesis at the level of light-harvesting could be mediated by the redox state of the plastoquinone pool, reduced plastoquinone serving to activate a protein kinase that catalyses phosphorylation of the major chlorophyll-containing protein complex. Information is now needed on whether this process is important in the physiological control of photosynthesis *in vivo*.

Acknowledgements

We wish to thank Dr J. Bennett for sending a copy of a manuscript prior to publication, and Dr J. Allen for communicating to us results that gave rise to the same conclusions as presented here. This work is supported by a grant from the Science Research Council.

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